Sterilization D Values

Sterilization by steam consist of the simple observation that bacteria die over time during exposure to heat. They do not all live for a finite period of heat exposure and then suddenly die at once, but rather at constant heat input, they cease to be alive in direct proportion the starting number of colony forming units (CFU). Where N is the number of CFU at any time t, we have the relationship $\frac{d}{dt}N = -k \cdot N$.

Somewhat obviously, the rate constant, k, is a function of the amount of heat put into the population, and thus it is a true constant only at constant heat input. In a steam autoclave, heat input is indirectly measured as temperature and pressue under known conditions of steam saturation (not to imply "saturated steam"). Shown below are the classical mathematics to quantify susceptibility of each species and strain of microorganism to heat.

$\frac{d}{dt}N(t) = -k \cdot N(t)$.......................Eqn 1) The primary differential equation.

$$\int_{N_0}^{N} \frac{1}{N(t)}\, dN(t) = \int_0^t -k\, dt$$

(Eqn 2) Solution by separation of variables for integration

$$\ln\left(\frac{N(t)}{N_0}\right) = -k \cdot t$$

(Eqn 3) The integrated result

$$\frac{N(t)}{N_0} = e^{-k \cdot t}$$

(Eqn 4) A simplification for understanding N(t)

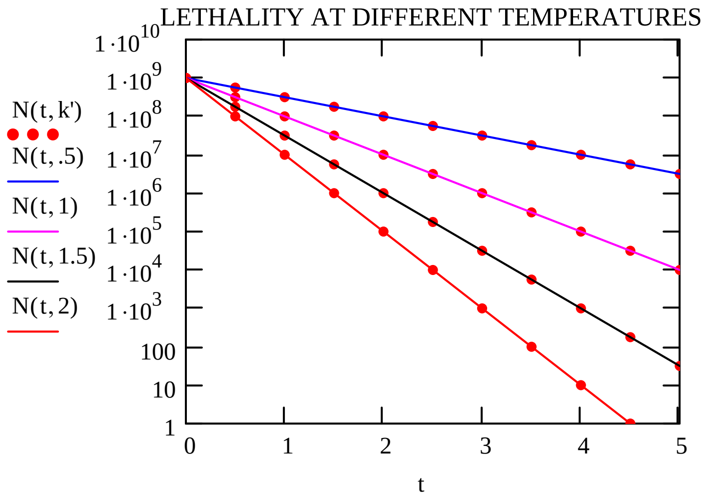
$$N(t) = N_0 \cdot e^{-k \cdot t}$$

Obviously, if the logarithmic base were changed from e to 10, the effect would be to have a different value for the constant k. [k' = k/ln(10)] Starting with 10^9 organisms, and a k' value between .5 and 2, the graphs are shown below.

$N_0 := 10^9$

$N(t,k') := N_0 \cdot 10^{-k' \cdot t}$ (Eqn 5)

$k' := .5, 1 .. 2 \qquad t := 0, .5 .. 5$



The constant, k, has units of min-1, since k*t must be unitless. Biologist have traditionally reported a value "D", which is the time required for a 10 fold reduction of CFU.

$D = \frac{1}{k'}$ So, in the graph shown, D=2 is the uppermost line with the least slope, i.e. the most difficult strains to sterilize, while D=0.5 is the lowermost line.

Putting D into equation 5 in place of k results in $N(t,D) := N_0 \cdot 10^{\frac{-1}{D} \cdot t}$

Solving for D, and remembering that D (a rate constant) is a function of temperature, T, we obtain the following.

$$D(T) = \frac{-t}{\log\left(\frac{N}{N_0}\right)} \qquad D = \frac{1}{k}$$

D has units of time (usually minutes), and it is the time required to reduce the microbiological populaton by 90%. From equation (3) above, we can obtain a ratio representing the survival of 10% of the organisms, i.e. 90% lethality.

$$\log\left(\frac{N(t)}{N_0}\right) = -k' \cdot t = \log(0.1)$$

$\frac{\log(0.1)}{-k'} = \frac{1}{k'} = D = t_{90}$ The D Value is the time for 90% of the organisms to be killed.

An explanation of z

Experience has shown that 250 deg F is a suitable temperature for the steam sterilization of many microorganisms. However, autoclaves may run hotter or colder. The question arises as to how to relate time at an actual autoclave temperature to time at a reference temperature.

How many degrees of temperature would be required to make D vary by 10 fold? For any given microorganism the question can be answered and the answer will be generically called "z".

That is, **z** represents a 10 fold change in D.

$$\frac{D(Temp0)}{D(Temp)} = 10 \qquad \frac{D_0}{D(T)} = 10$$ <= Note the log(10) = 1

Simultaneously, z represents the temperature difference between T_0 and T.

$$z = T - T_0$$

$$\log\left(\frac{D_0}{D(T)}\right) = 1 \qquad 1 = \frac{T - T_0}{z}$$ Where D_0 is the D value determined at T_0.

$$\log\left(\frac{D_0}{D(T)}\right) = \frac{T - T_0}{z}$$

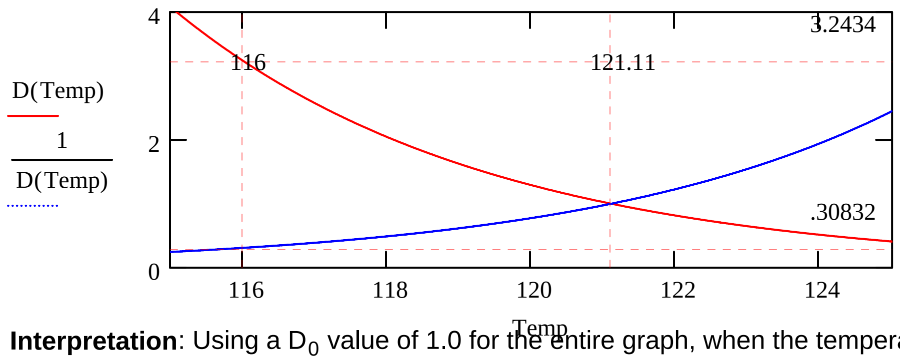
$$\frac{D_0}{D(T)} = 10^{\frac{T-T_0}{z}}$$

$$\frac{1}{D(T)} = \frac{1}{D_0} \cdot 10^{\frac{T-T_0}{z}}$$ <= this expression would have units of inverse minutes, since D has units of minutes.

$z := 10 \quad T_0 := 121.11 \quad D_0 := 1.0$

$$D(Temp) := \frac{D_0}{10^{\frac{Temp-T_0}{z}}}$$

D is the time required, calculated for any temperature, T, to annihilate 90% of the population. When D_0 = 1min, the reference temperature (here 121.11 deg C) is needed for t_{90}.

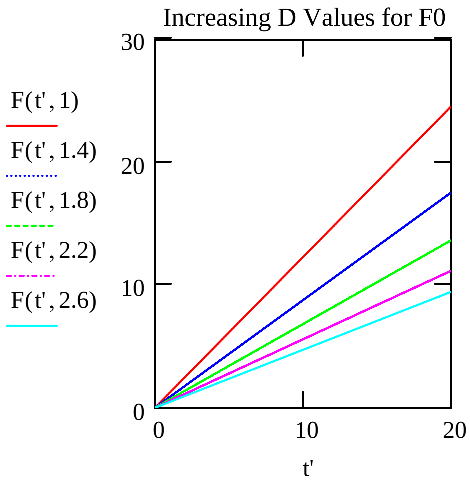


Interpretation: Using a D_0 value of 1.0 for the entire graph, when the temperature is 116, there will be required 3.24 minutes to reduce the population by 90%. Conversly (or inversely) in 1 minute at 116 C, only 0.308 minutes of equivalent lethality are accumulated.

But now we have a function that relates lethality(90% annihilation), D_0, and Temp, and can be integrated to obtain time. $F(T) = \int_{t_0}^{t} \frac{1}{D_0} \cdot 10^{\frac{T-T_0}{z}} \, dt = \frac{(t - t_0)}{D_0} \cdot 10^{\frac{T-T_0}{z}}$ This integration result assumes a constant temperature T for the entire period from t_0 to t. If T(t) is a function of time, i.e. if Temperature varies with time, as it almost certainly will, then a specific integral must be written after the temperature funtion is known. In most cases, there is no math function for the variation of Temperature with time, and thus a numerical integration will be needed.

$t_0 := 0$ $z := 10$ $T_0 := 121.11$ $T := 122$

$$F(t, D_0) := \frac{(t - t_0)}{D_0} \cdot 10^{\frac{T - T_0}{z}}$$



The integral function is used by microbiologist and referred to as F_0 , often without the $1/D_0$ term which is assumed to have a value of 1.0 min. When used with D_0, F_0 is unitless since time in minutes (dt) over D_0, also in minutes, cancel each other.

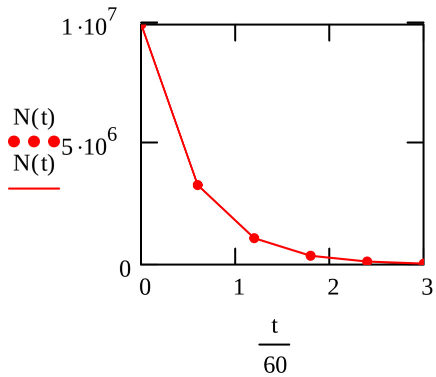
$$F_0 = \frac{1}{D_0} \cdot \int_{t_0}^{t} 10^{\frac{T - T_0}{z}} \, dt$$

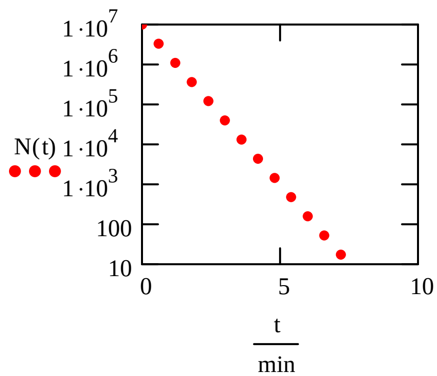
Its primary use is in relating sterilization temperature and time with one microorganism back to a standard sterilization temperature and time. F_0 is usually used as the equivalent number of minutes at 121.11 T_0, that are counted as a result of the Temperature at which the system is measured.

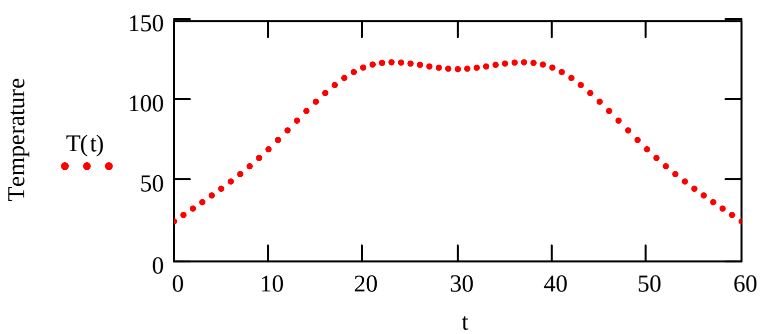
Example:

$t := 0min, \frac{60}{10^2}min .. 60min$ $N_0 := 10^7$ <= Starting number of microorganisms.

$k' := \frac{.8}{min}$ $N(t) := N_0 \cdot 10^{-k' \cdot t}$ $\frac{1}{k'} = 1.25\,min$







$D_0 := 1$

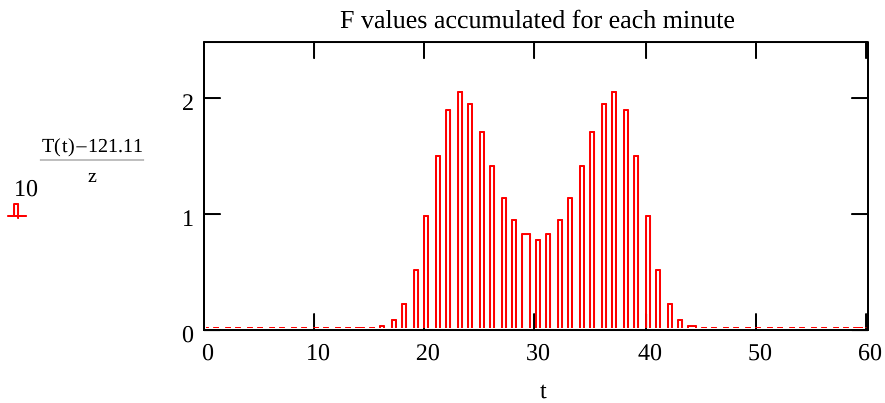
Temperature time trace from a steam sterilizer, using simulated data, T(t).

$z := 10$

$$\frac{1}{D_0} \cdot \left(\int_{20}^{40} 10^{\frac{T(t)-121.11}{z}} \, dt \right) = 28.681$$

$$L_j := 10^{\frac{T(j)-121.11}{z}}$$

$10^{\frac{T(t)-121.11}{z}}$



For nearly flat Temperature time traces such as the one shown, between 20 and 40 minutes, the integral is sometimes evaluated using a sum over evenly timed increments.

$$\frac{1}{D_0} \cdot \sum_{t=20}^{40} 10^{\frac{T(t)-121.11}{z}} = 29.567$$

This is a calculation method for integration. There are many different methods of numerically obtaining the integral, hence there will be small differences in the values. These differences should be insignificant in all cases.

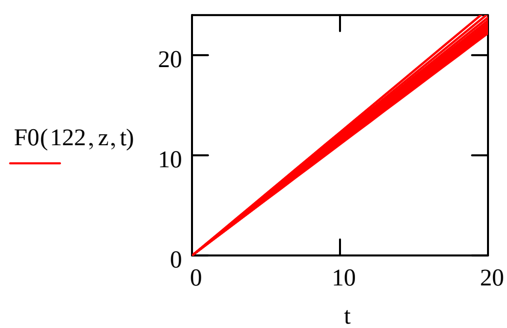
It is also possible to integrate over the entire time period, thus getting Lethality accumulation for the up and down times, getting to and from the sterilizing temperature plateau.

$$\frac{1}{D_0} \cdot \int_0^{60} 10^{\frac{T(t)-121.11}{z}} \, dt = 31.25$$

$$\sum_{t=0}^{60} \frac{1}{D_0} \cdot 10^{\frac{T(t)-121.11}{z}} = 31.25$$

When the temperature,T, is close to 121.1, influence from the value of z between 10 and 20 is very limited. Clearly, when T matches the reference Temperature, then $10^{\frac{0}{z}} = 1$ no matter what value z may assume.

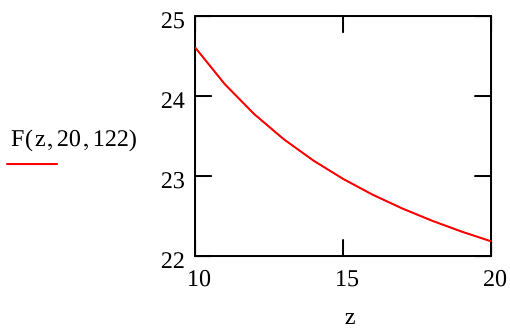
$$F0(T,z,t) := \frac{1}{D_0} \cdot \int_0^{t} 10^{\frac{T-121.1}{z}} \, dt \qquad z := 10, 11 .. 20$$



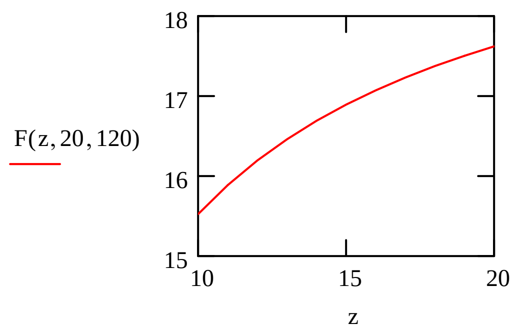
Indeed, when the Temperature is greater than the reference temperature (121.1 above), then accumulated F_0 decreases when z increases, but when the Temperature is less than the reference, accumulated F_0 increases when z increases.

$$F(z,t,T) = \frac{1}{D_0} \cdot \int \frac{10^{T-121.1}}{z}\, dt \qquad F(z,t,T) := \frac{1}{D_0} \cdot 10^{\left[\frac{(T-121.1)}{z}\right]} \cdot t$$

Effect of a change in z value on Accumulated Lethality, F.



T=122 > TRef = 121.1



T=120 < TRef = 121.1